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Are CRISPR/Cas genome editing techniques the future of plant breeding?

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ABSTRACT

CRISPR/Cas-based innovative breeding technologies now provide plant breeders with unprecedented opportunities to produce genetic variation for breeding. The ability to effectively target changes in most crops thanks to recent advancements in CRISPR/Cas genome editing suggests that agricultural progress may quicken.

Keywords: CRISPR/Cas9, Genome editing, Plant breeding, Wheat, Rice

The gene editing (GE) technology CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein), commonly known as the "genetic scissors," was first published 11 years ago after its establishment by Emmanuelle Charpentier and Jennifer Doudna (Jinek *et al.,* 2012). If ethical concerns are taken seriously, the application of CRISPR/Cas technology could be revolutionary in many fields where therapeutic applications are at the forefront. Doudna and Charpentier were awarded the Nobel Prize in Chemistry in 2020 for their significant contribution to developing a technology that facilitates "rewriting the code of life," according to the statement of the secretary-general of the Royal Swedish Academy of Sciences. CRISPR/Cas9 is currently the most common editing system for plant genomes (Impens *et al.,* 2022), owing to the fact that it requires only the expression of generic Cas9 endonuclease and one (or more) single guide RNAs (sgRNAs) specially designed to match part of the sequence of target gene positions, allowing it to significantly and precisely chop certain DNA sequences. The era we are living in is marked by an unprecedented growth rate of the global human population. The current estimated world population is 7.7 billion and is expected to soar to 8.8 billion by 2030 and approximately 10 billion by 2050 (Bhatta and Malla, 2020). This challenge triggered an unpleasant demand for higher amounts of food (≈50%) which imposes severe burdens on current limited agricultural productivity. Climate change exacerbates the situation by increasing atmospheric temperature, increasing drought, and increasing soil salinity, all of which reduce global agricultural productivity and threaten food security (Hazman *et al.,* 2022). Additionally, climate change was found to make plants more vulnerable to pests and pathogens, which substantially negatively impacts crop yield and quality (Kim *et al.,* 2022). As a result, the most effective strategy for bridging this gap is for every land area unit (for example, an acre) to become more productive.

In this context, researchers worldwide have made significant efforts to sustain agricultural production in the face of the climate crisis and its new normal environmental challenges. Plant conventional breeding is one of the most widely accepted sustainable strategies for achieving climate-resilient crops, yet it consumes extensive resources and time. Other methods were adopted under the auspices of modern agriculture to increase agriculture productivity, such as mutation breeding and transgene breeding; nevertheless, both required a similarly extended period (8–12 years) and are characterized by a high level of uncertainty in terms of achieving precision genome engineering. Identifying the few individuals with a desirable characteristic from a huge population of mutagenized plants, on the other hand, is labor-intensive and time-consuming (Fig. 1). Also, government restrictions oversee both cross-breeding and transgenic breeding. Furthermore, negative public perceptions about the safety of these products limit their potential (Gao *et al.,* 2021).

On the other hand, transgenic-free CRISPR/Cas9 technology was able to produce an elite variety by targeting specific genes within a shorter period (4-6 years) (Chen *et al.,* 2019; Abd-Elsalam and Lim, 2021; Alghuthaymi *et al.,* 2021). Enhancing crop productivity within a short period to efficiently keep pace with expanding food demand will be a big challenge without an efficient plant genome engineering strategy. Because all types of crops (cereals and horticultural) are required to ensure human and livestock food security, recent gene-editing technologies such as CRISPR/Cas9 have been used to improve yield in terms of quality (nutritional value) and quantity. In the area of agriculture, CRISPR/Cas9 was beneficial in terms of (i) basic science trends for understanding gene function and (ii) generating new genotypes with enhanced traits and breeding application potential.

CRISPR/Cas9 was utilized to investigate molecular mechanisms in plants; for example, Ortigosa et al. (2018) could utilize CRISPR to study the role of a photoreceptor in seedling development/stress tolerance in tomato, and Martín-Pizarro et al (2019) could characterize transcription factor involved in another development in strawberry. At the level of assessing gene regulation, CRISPR/Cas9 could be successful in manipulating gene expression by controlling promoters without the need to insert foreign DNA in lettuce and Arabidopsis (Zhang *et al.,* 2018). Indeed, the ability of CRISPR/Cas9 to knockout/in certain genes using free foreign-DNA techniques would discharge possible biohazard burdens issues and thus facilitate the marketing of CRISPR/Cas9-produced crops (Zhang *et al.,* 2020).

In 2013, the first successful genome editing trial was achieved by Upadhyay et al. (2013) in wheat and *Nicotiana benthamiana* which proved that CRISPR/Cas9 is functional in plants. Ever since CRISPR/Cas9 technology started to be considered the most popular genome editing technology used for crop genome engineering by enhancing the following aspects: broadening genetic variability for plant breeding, stress tolerance, crop yield quantity and quality, and in synthetic biology. The intrinsic potential of plant breeding relies on the availability of variable genetic resources with different valuable traits. The traditional method for securing plant genetic variability as natural resources, chemical or irradiation mutagenesis are time and resources consuming. CRISPR/Cas9, on the other hand, could be utilized to broaden genetic resources by knocking out/knocking-in mutations (single, duplex, or multiplex), manipulating gene expression, and encouraging meiotic crossover (Veillet *et al.,* 2020). Producing stress tolerant and pathogen-resistant plants by CRISPR/Cas9 was demonstrated to be an efficient trend. As an example: precise knocking out MdDIPM4 in apple and destroying MLO allele in tomato led to achieving resistant lines to the bacteria *Erwinia amylovora* and powdery mildew fungi, respectively (Pompili et al., 2020; Nekrasov *et al.,* 2017). Target editing rice genes OsGn1a, OsDEP1, and OsGS3 enhanced grain number, dense erect panicle, and grain size, respectively (Li *et al.,* 2016). In maize, the CRISPER/Cas9 technology was used to improve the industrial value of corn starch (adhesive and high glossy paper production) by inhibiting the biosynthesis of another long-chain polysaccharide amylose by knocking out Wx1 gene, the process that exclusively sharpened the biosynthesis of branched polysaccharide amylopectin (Waltz, 2016). The main concept in synthetic biology is to re-design any plant species to be able of producing certain biological components that do not exist, therefore, CRISPR-based genome editing systems could pave the way for a revolutionized era of synthetic biology. In this context, any CRISPR-produced crop could be classified as a synthetic biology approach. According to Schachtsiek and Stehle (2019), CRISPR/Cas9 technology could be utilized to help people overcome nicotine addiction by producing nicotine-free non-transgenic tobacco.

Although there has been a continuous application of CRISPR/Cas9 technology in agriculture since 2013, there are few CRISPR-produced crops in the market. As with any other technology, there might be several technical obstacles such as target limitation, size of the catalytic window, and off-target editing (Mishra *et al.,* 2020), however, this is not the real challenge for genome editing-based crops. The main issue is thought to be the market where customers need more assistance to trust and thus support gene-edited crops. Such trust-building is far from trivial, yet could be handled by providing the public with reliable information about CRISPR technology and its possible impact. Equally importantly, genome editing regulations should be issued dependably and responsibly, i.e., encourage the emerging genome-editing technology (especially versus GMO) yet it seriously respecting public health and environmental or other agricultural ecosystem stability. In this regard, there are great variations between countries. Indeed, the European Court of Justice ruled that gene editing technologies must be subjected to GMO regulation, a prevented commodity in the EU (Hjort *et al.,* 2021). On the other hand, other countries such as the US and Canada where GMO crops were already cultivated for a long period, passed genome-editing legislations without any further specific terms and conditions. It is worth mentioning that relevant debates generated in response to GMOs are still hampering the legislation of genome editing crops in many other countries. Therefore, clear and scientific discussion panels should be legally organized by specialized scientists to strengthen the link between the public and legislation authorities. Finally, CRISPR/Cas-based innovative breeding technologies now provide plant breeders with unprecedented opportunities to produce genetic variation for breeding. The ability to effectively target changes in most crops thanks to recent advancements in CRISPR/Cas genome editing suggests that agricultural progress may quicken.

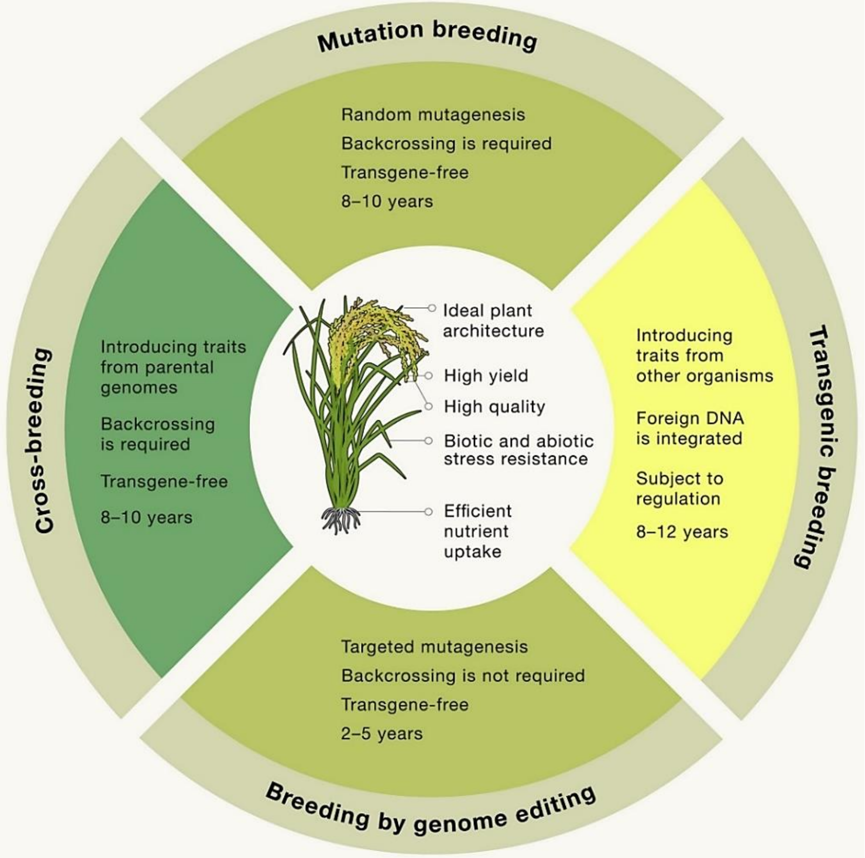

Fig. 1. Different Plant breeding methods employed to produce and improve crop cultivars (Reprinted from Gao, 2021).

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